has received very little focus (for an exception, see Pessiglione et al., 2008).

The current findings also expose new and interesting questions. Predominantly, this behavioral study invites a neural examination of the elements involved in unconscious reward learning. For example, where does the neural plasticity occur which enables the observed unconscious perceptual learning? The cortex would appear to represent the most likely site, but the fact that learning was largely specific to the trained eye suggests that the changes occur very early on in the sensory pathways, before visual information from both eyes converges. Moreover, are the neural changes present during unconscious perceptual learning qualitatively different from those involved during learning under conditions of awareness? A prosaic possibility is that learning under these two conditions acts on the same neural circuits but to a greater or lesser extent according to the differing perceptual strength of the stimuli. Another question is by what neural mechanisms are the plasticity changes underlying such learning accomplished. The neuromodulator dopamine has been characterized as playing a key role in learning about rewards (Schultz et al., 1997), but dopamine is not known to have strong projections to sensory cortical areas, so is perhaps unlikely to have involvement in the sensory aspects of learning during conditioning. On the other hand, the neurotransmitter acetylcholine is thought to be involved in sensory learning (Weinberger, 1995), although it is also suggested to contribute to the control of attention (Yu and Dayan, 2005).

On a cautionary note, there are some who contend that learning without awareness has not been convincingly demonstrated (Lovibond and Shanks, 2002) by challenging the extent to which behavioral assays have succeeded in verifying that subjects are truly unaware of stimulus contingencies. A conciliatory position might be that conscious and unconscious learning is better viewed not as a dichotomy but rather a continuum along which stimuli vary in the extent that they activate sensory representations. Regardless, it is clear that studies such as the present which probe the boundaries of human learning will help to provide new behavioral tools for the study of the neural mechanisms underlying reward, learning, attention, and their interactions.

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Synaptogenic Proteins and Synaptic Organizers: "Many Hands Make Light Work"

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Synaptogenesis is thought to be mediated by cell adhesion proteins, which induce the initial contact between an axon and its target cell and subsequently recruit and organize the presynaptic and postsynaptic protein machinery required for synaptic transmission. A new study by Linhoff and colleagues in this issue of *Neuron* identifies adhesion proteins of the LRRTM family as novel synaptic organizers.

The formation of synapses between neuronal axons and target neurons proceeds in two phases. After an axonal growth cone has reached its correct target area, it first has to select its appropriate synaptic partner from a large number of different types of neurons and neuronal processes. This initial phase of cell-type-specific target recognition is followed by a synapse maturation phase, during which the protein components of the presynaptic release machinery and the postsynaptic signaling apparatus are recruited to the nascent synaptic contact. Both phases of synaptogenesis are

thought to be regulated by cell adhesion proteins (Yamagata et al., 2003).

The hypothesis that cell-type-specific synaptogenesis and the formation of neuronal networks require a chemoaffinity code of adhesion proteins was put forward over 40 years ago (Sperry, 1963). However, the search for these adhesion proteins in the mammalian central nervous system has been very difficult. It took several decades until a first major breakthrough was achieved, in the course of which it was shown that presynaptic specializations are induced in axons of neurons that had been cocultured with fibroblasts expressing postsynaptic cell adhesion proteins of the neuroligin family (Scheiffele et al., 2000).

Based on this observation, it was suggested that neuroligins are key triggers of synapse formation in the mammalian brain, which was supported by the subsequent finding that neurexins, presynaptic adhesion proteins that bind to neuroligins, trigger postsynaptic specializations in the neuron-fibroblast coculture assay (Graf et al., 2004). But things were complicated rapidly as several other cell adhesion proteins were found to also have synaptogenic activity in the same coculture assay, including SynCAMs/Necls (Biederer et al., 2002), EphBs and ephrinBs (Aoto et al., 2007; Kayser et al., 2006), and netrin G ligands (NGLs/LRRC4s; Kim et al., 2006).

With a beautiful and comprehensive new study, the group of Ann Marie Craig, in collaboration with the groups of Stephen Strittmatter and Matti Airaksinen, now adds a fascinating new cell adhesion protein family to this list, the LRRTMs (Linhoff et al., 2009 [this issue of *Neuron*]). The authors used the neuron-fibroblast coculture assay that had previously been used to test the synaptogenic activities of candidate proteins in order to perform an expression screen for synaptogenic proteins from rat brain. They rediscovered NGL-3/LRRC4B and neuroligin-2, which nicely validated their screening approach, and found a cDNA clone encoding LRRTM1 that causes overexpressing fibroblasts to induce presynaptic specializations in cocultured neurons. LRRTM1 is a member of a family of four type 1 transmembrane adhesion proteins with 10 extracellular leucine-rich repeats that mediate protein-protein interactions, and a short C terminus that ends in a class I PDZ-domain-binding motif.

Further analyses showed that all four LRRTMs induce the differentiation of glutamatergic presynaptic specializations in the coculture assay. This finding, together with the observation that LRRTM1 and LRRTM2 are specifically localized to excitatory glutamatergic synapses, indicates that LRRTMs are specific for glutamatergic synapses. That LRRTMs can trigger synaptogenesis in neurons was demonstrated by the fact that overexpression of LRRTM2 caused an increase of the number of presynaptic terminals targeting the overexpresing neuron.

Thus, LRRTMs are sufficient to trigger the formation of excitatory synapses, like members of the neuroligin, neurexin, SynCAM/Necl, EphB, ephrinB, and NGL/LRRC4 protein families. But are they necessary for initial synapse formation in vivo? Although this question could not be addressed comprehensively in the new study on LRRTMs because this would require perturbation or deletion of all four LRRTMs at once, a first data set indicates that LRRTMs may not be necessary for synapse formation in the brain. Genetic deletion of LRRTM1 in mice did not affect the total number of synapses in the synaptic neuropil of the hippocampus. The only change reported in the study was an increase in the presynaptic area occupied by VGLUT1 in some strata of the hippocampal neuropil of the CA1 region, with apparently normal active zone integrity, indicating a role for LRRTM1 in the selective recruitment or anchoring of synaptic vesicles.

As already stated, the parallel perturbation or deletion of all four LRRTMs will be necessary to determine unequivocally whether LRRTMs are required for initial synapse formation. Thus, LRRTMs may still turn out to be key triggers of synaptogenesis. Indeed, a recent study examining synapse formation between motor neuron axons and body wall muscles in *Drosophila* identified several leucine-rich repeat proteins as key mediators of synaptic target selection (Kurusu et al., 2008). On the other hand, the authors of the new LRRTM paper state that "proteins testing positive for synaptogenic activity in [the neuron-fibroblast coculture] assay may not be essential for initiating the formation of or maintaining the integrity of synaptic junctions" and go on to argue that "these factors may [rather] serve a role in the maturation of the synapse, recruiting components necessary for synaptic function" (Linhoff et al., 2009). This assessment is supported by a systematic genetic study, in which the role of neuroligins, a protein family that is synaptogenic in the neuron-fibroblast coculture assay, has been examined in vivo. It showed that the complete elimination of all neuroligins in the mouse brain does not affect synapse numbers but alters the recruitment of postsynaptic receptors to glutamatergic, GABAergic, and glycinergic synapses, which demonstrates that neuroligins are required for synapse maturation but not for the initiation of synaptogenesis (Varoqueaux et al., 2006). To complicate things further, the synaptic function defects in neuroligin-deficient neurons in vivo are not detectable in cultured neurons (Varoqueaux et al., 2006; Chubykin et al., 2007), which leads the authors of the new study on LRRTMs to suggest that "the function of … synaptic organizing molecules may be assessed accurately only by in vivo studies" (Linhoff et al., 2009).

The authors' statements (Linhoff et al., 2009) quoted above summarize the key aspects of what needs to be done next. The LRRTM1 knockout phenotype is already being analyzed in more detail, which promises to be informative, and knockouts of the other LRRTMs are under way. A comprehensive genetic analysis of the function of the LRRTM protein family is therefore within reach. Beyond studies on the cell biological role of LRRTMs, an analysis of the behavioral phenotypes of LRRTM knockouts will also be interesting because the human *LRRTM1* gene appears to be associated with handedness and schizophrenia (Francks et al., 2007). While it may be difficult to study handedness in mice (Sun and Walsh, 2006), behavioral correlates of schizophrenia can be examined in mice (Flint and Shifman, 2008), and the LRRTM1 knockouts may turn out to be a useful model in this regard.

Another important issue that needs to be addressed in the future concerns the exact synaptic localization of LRRTMs and their synaptic binding partners. Endogenous LRRTM2 is localized to glutamatergic postsynaptic sites and

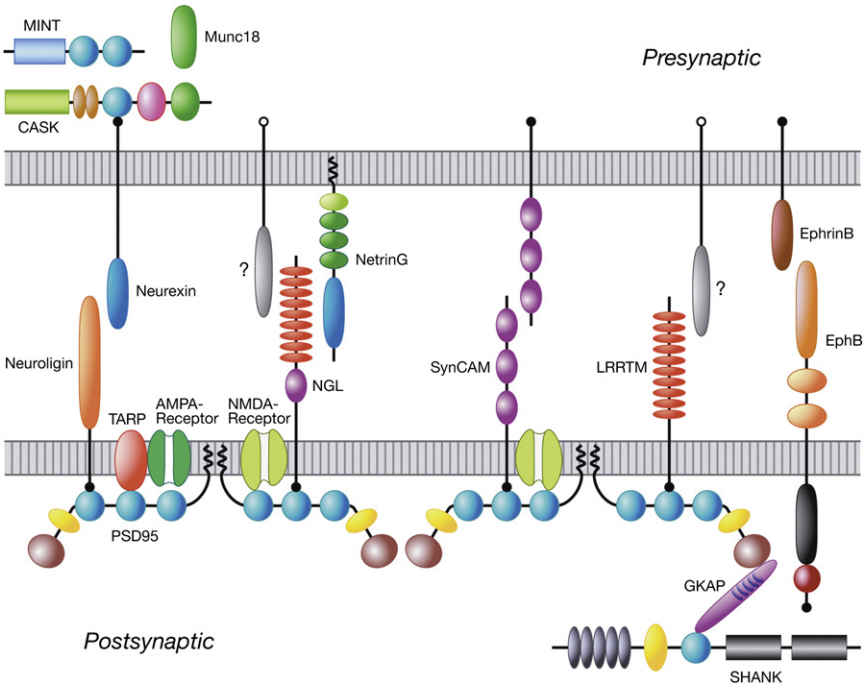

Figure 1. Multiple Adhesion Systems at Glutamatergic Synapses
The image depicts synaptic cell adhesion systems that show synaptogenic activity in the neuron-fibroblast coculture assay (see text for details). Note that several additional adhesion proteins were shown to be present at this type of synapse and to contribute to synapse formation and stabilization (e.g., SALMs, Nectins, and N-cadherin).

YFP-LRRTM1 seems to behave similarly, yet LRRTM1 knockout neurons exhibit a presynaptic defect in synaptic vesicle clustering. Thus, LRRTMs can signal *trans*-synaptically, but the underlying mechanism remains enigmatic. LRRTM2 does not seem to engage in homophilic interactions, which indicates that it may interact *trans*-synaptically with as yet unidentified presynaptic adhesion proteins. The identification of these presynaptic binding partners of LRRTMs is a key prerequisite for understanding LRRTM function. It will also shed light on the observations that LRRTM2 is enriched in certain strata of the hippocampal neuropil and that the deletion of LRRTM1 affects synaptic vesicle clustering in hippocampal neurons in a stratum-specific manner.

From my point of view, the most striking conclusion that can be drawn from the new paper on LRRTMs (Linhoff et al., 2009) and previous studies on synaptic adhesion proteins is that individual glutamatergic synapses in the mammalian brain contain multiple adhesion systems that operate in parallel (Figure 1). Among these, adhesion proteins containing leucine-rich repeats such as LRRTMs or NGLs/LRRC4s are prevalent. Significant functional redundancies exist between members of the same protein families. This is, for example, illustrated by the rather subtle phenotypic changes in individual neuroligin knockouts (Varoqueaux et al., 2006; Chubykin et al., 2007), which cumulate in compound knockouts of multiple neuroligin isoforms (Varoqueaux et al., 2006), or by the fact that different LRRTM isoforms are targeted to the same types of synapses (Linhoff et al., 2009), which is also seen for other families of synaptic cell adhesion proteins. It is very likely that such redundancies also exist between the different types of adhesion systems at glutamatergic synapses. The corresponding adhesion proteins are often colocalized at synapses and many of them interact with the same intracellular scaffold proteins.

In view of these considerations, it is probably wrong to assume that the cell-type-specific initiation phase of synaptogenesis or key steps of synapse maturation are determined by individual adhesion partners in the mammalian brain. Instead, combinations of the different adhesion systems and their permutations may trigger and instruct cell-type-specific synaptogenesis, allow for synapse diversity, and guarantee the robustness of subsequent synapse maturation and stabilization. Thus, as far as the role of adhesion systems at mammalian glutamatergic synapses is concerned, it does not seem that too many cooks spoil the broth. Rather, *mani hondes maketh light werk*, as it was put in the medieval English romance "Sir Beves of Hamtoun."

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